

ELECTRICAL INDUCTION OF SPAWNING IN TWO MARINE
INVERTEBRATES (*URECHIS UNICINCTUS*,
HERMAPHRODITIC *MYTILUS EDULIS*)

YASUO SUGIURA

Misaki Senior High School, Miura-Shi, Kanagawa Prefecture, Japan

The investigations of Iwata (1950) and Harvey (1952) have proved electrical stimulation to be a very effective means for inducing spawning of sexual cells in sea urchins. Recently, Kubota (1962) has reported the same effect in an insect (dragon-fly). Inspired by their success, the author tested the applicability of this method to various shore invertebrates, two successful cases of which will be briefly reported.

I. *Urechis unicinctus*

MATERIALS AND METHODS

By the ordinary practice, for obtaining reproductive cells of *Urechis*, either the body wall must be cut open or the eggs must be collected directly from the opening of the gonoduct by a fine pipette (Newby, 1932). When the electrical method is used, it is possible to obtain with ease any desired quantity of reproductive cells, and this can be applied repeatedly to the same individual, if necessary, since spawning lasts only during the stimulation. Moderate stimulation of the adults causes no harmful effects in the later development of the gametes.

The spawning season of *Urechis unicinctus* is said to be from October to March (Hiraiwa and Kawamura, 1936; Ohkawa, 1958; Sakiyama, 1958). At Kisarazu, Chiba Prefecture, where this study was performed, the rate of occurrence of sexually mature individuals remains fairly high even in April. Twenty animals (about 10 cm. in length in contracted state) were used.

One individual at a time is placed in a vessel (155 mm. in diameter, 33 mm. in depth) filled with sea water, and a pair of Ag-electrodes (1 mm. in diameter) are dipped vertically into the sea water 20 mm. apart across the body axis of the worm. The temperature and the specific gravity of the sea water are adjusted to 16.0–20.0° C. and 1.0209–1.0232, respectively, and alternating current (30 volts) is applied for various periods.

RESULTS

As soon as the stimulation is applied, the animal contracts quickly and in many cases a large swelling appears in the front of the body. About 4–20 seconds after stimulation, the animal begins to discharge reproductive cells forcibly through the openings of the gonoducts (Fig. 1). There is no conspicuous difference between females and males in their reaction to electrical stimulation.

On cessation of stimulation, the animal relaxes and after a while spawning also stops, a mass of sexual cells being deposited on the bottom of the vessel. A con-

siderable amount of eggs or sperm can be obtained by a single discharge ranging from 5 to 35 seconds.

Over-stimulation lasting more than 30 seconds should be avoided, since this causes prolonged contraction or occasionally even death. The time required for recovery from the stimulation-induced contraction varied widely, from several minutes to several hours. Intermittent stimulations of very short duration seem to act excessively: after three stimulations of 6 seconds each, given 3-5 minutes apart, although all the animals discharged, 20% of them failed to recover from contraction.

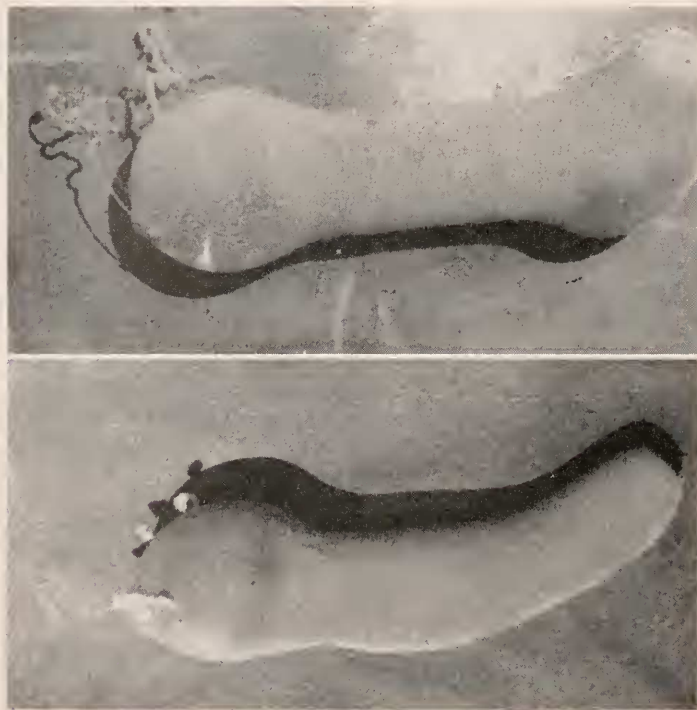


FIGURE 1. *Urechis uncinatus* spawning under electrical stimulation.

If due caution is paid to the duration of, and the intervals between, stimulations, one to two stimulations a day can be given for 6 consecutive days to animals fully charged with reproductive cells. In one case, animals which had been kept for 11 days in captivity and made to spawn nine times, still discharged eggs which developed on insemination, to normal trochophores.

II. Hermaphroditic *Mytilus edulis*

MATERIALS AND METHODS

Mytilus edulis is normally dioecious, and hermaphroditic individuals can be found only very rarely. Among some 400 individuals examined by the author at

Kisarazu, Chiba Prefecture, four hermaphroditic mussels were met with (Fig. 2). They were detected by the colour of the gonads and the condition of the follicles, and further checked by smear preparations of the gametes.

With respect to the induction of spawning by electrical stimulation of *Mytilus*, Iwata's report is available (1949). In 1951, he further showed that discharge could be induced in excised parts of the mantle. Since *Mytilus* eggs are unfertilizable as taken from excised ovaries, requiring to be at least quasi-normally spawned, this method of Iwata's was used to obtain gametes from such hermaphroditic individuals.

Pieces of the mantle about 5 mm.², containing either ovary or testis, were cut out of a hermaphroditic individual. Each piece was stimulated separately by 40 volts a. c. for 15 seconds with a pair of Ag-electrodes 1 mm. in diameter, placed 20 mm.

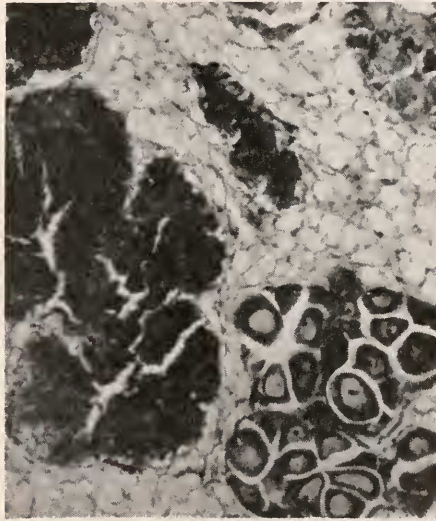


FIGURE 2. Hermaphroditic gonad.

apart in a vessel of 72 mm. diameter filled with sea water to a depth of 5 mm. After a latent period of about 30 minutes (19° C.), eggs and sperm were spawned; these were used for cross-fertilization in various combinations. In no case did eggs to which sperm was not added show any development, indicating that they were not contaminated with sperm during spawning.

RESULTS

Self- and cross-fertility

Separately obtained gametes were mixed in various combinations and the percentages of resulting fertilization were counted. The results are summarized in Table I. Later development of such zygotes was followed; in all four hermaphroditic individuals, self-fertilization zygotes were found to develop at the normal pace, and the embryos showed no difference whatsoever from out-bred controls.

Gametes were sometimes obtained from an ovary and a testis situated in the mantles of opposite valves and in other cases they occurred together on the same side;

TABLE I
Fertilizability of gametes from hermaphroditic mussels

Combination	Fertilization percentage
Herm. egg × herm. sperm	92.8%
Normal egg × herm. sperm	94.0%
Herm. egg × normal sperm	93.2%
Normal egg × normal sperm	96.0%

in both cases the results were identical. In one mussel, a piece of the mantle containing gonads of both sexes was stimulated and normal larvae were obtained without further insemination.

These facts lead to the conclusion that the gametes of hermaphroditic *Mytilus edulis* are capable of self-fertilization which produces perfectly normally developing larvae.

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SUMMARY

1. Electrical stimulation of spawning was tried on *Urechis unicinctus* and hermaphroditic *Mytilus edulis*.

2. *Urechis* reacts immediately, and if over-stimulation is guarded against, samples can be repeatedly obtained from the same individual, which would be very advantageous for some kinds of experiments.

3. Excised pieces of hermaphroditic *Mytilus* were stimulated electrically to spawn; the gametes so obtained showed perfect self-fertility.

LITERATURE CITED

- HARVEY, E. B., 1952. Electrical method of "sexing" *Arbacia* and obtaining small quantities of eggs. *Biol. Bull.*, **103**: 284.
- HIRAIWA, Y., AND T. KAWAMURA, 1936. Relation between maturation division and cleavage in artificially activated eggs of *Urechis unicinctus*. *Biol. Bull.*, **70**: 344-351.
- IWATA, K. S., 1949. Spawning of *Mytilus edulis*. (2) Discharge by electrical stimulation. *Bull. Jap. Soc. Sci. Fish.*, **15**: 443-446. (in Japanese)
- IWATA, K. S., 1950. A method of determining the sex of sea urchins and of obtaining eggs by electric stimulation. *Annot. Zool. Jap.*, **23**: 39-42.
- IWATA, K. S., 1951. Spawning of *Mytilus edulis*. (5) A method to obtain mature eggs from mantle piece. *Bull. Jap. Soc. Sci. Fish.*, **17**: 15-18. (in Japanese)
- KUBOTA, T., 1962. An electrical method of forcing the damselfly (Odonata) to oviposit. *Zool. Mag.*, **71**. (in Japanese)
- NEWBY, W. W., 1932. The early embryology of the echiuroid, *Urechis*. *Biol. Bull.*, **63**: 387-399.
- OHKAWA, M., 1958. Studies on the fertilization in eggs of the echiuroid worm, *Urechis unicinctus*. (3) On the growth of egg-cells suspended in the body fluid. *J. Yokohama Municipal Univ. Ser. C-25*, **95**: 1-13. (in Japanese)
- SAKIYAMA, F., 1958. Rearing experiments of *Urechis* larvae. *Nat. Sci. Rep. Ochanomizu Univ.*, **9**: 47-56.